

Coagulation Changes to Systemic Acidosis and Bicarbonate Correction in Swine

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Background: As part of our overall interest in the mechanisms and treatment related to the development of the lethal triad of hypothermia, acidosis, and coagulopathy seen in trauma patients, the purpose of this study was to determine whether acidosis, inducible either by HCl infusion or hemorrhage/hypoventilation, leads to coagulopathy, and if correction of the acidosis will alleviate this coagulopathy.

Methods: In two separate experiments, acidosis was induced in anesthetized swine by (1) HCl infusion ($n = 10$) or (2) hemorrhage/hypoventilation ($n = 8$). Arterial blood samples were taken before HCl infusion or hemorrhage (arterial pH 7.4), after HCl infusion or hemorrhage (pH 7.1), and after bicarbonate infusion to return pH to 7.4. Arterial pH and blood gases were measured every 15 minutes.

Results: Acidosis (arterial pH 7.1) led to a hypocoagulation as measured by several coagulation parameters. In both experiments, acidosis was associated with a significant decrease in the maximum strength of the clot and the rate at which the clot formed. There was a significant decrease in endogenous thrombin potential and maximum thrombin concentration after acidosis in both groups (thrombin generation assay). However, the activated clotting time, prothrombin time, and activated partial thromboplastin time were significantly elevated only in the HCl-infused group. Fibrinogen concentration and platelet count were significantly reduced in both groups after acidosis. The hypocoagulation that was induced by either hemorrhage/hypoventilation or HCl infusion was not immediately corrected after returning pH to 7.4 with bicarbonate injection.

Conclusions: These data suggest that acidosis induced by HCl infusion or by hemorrhage/hypoventilation leads to hypocoagulation. Simple correction of the arterial pH with bicarbonate is not sufficient to correct this coagulopathy.

Key Words: Coagulopathy, Thromboelastography, Thrombin generation, Activated clotting time, Rotational thromboelastogram.

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Trauma is the leading cause of death and disability in the young and exceeds all other causes of death worldwide.^{1,2} Hemorrhage and trauma accounts for 35% to 40% of all

civilian trauma deaths and the majority of deaths on the battlefield.^{3–6} Severely injured trauma patients are cold, acidotic, and coagulopathic; show persistent bleeding; and are in need of continued transfusion, a condition referred to as the lethal triad.^{7,8} Resuscitation, with crystalloid, colloid, or packed red blood cells dilutes plasma coagulation factors and exacerbates any existing coagulopathy, leading to further blood loss.^{9,10}

Experimental evidence shows that acidic pH inhibits the clotting properties of blood, leading to hypocoagulation. In vitro acidification of plasma decreases fibrinogen concentration, and the activity of clotting factors V and IX.¹¹ A pH change from 7.4 to 7.0 decreased factor VIIa and factor VIIa-tissue factor complex activity by 90% and 60%, respectively.¹² In vivo, acidosis inhibits platelet aggregation,¹³ increases fibrinogen degradation rate,¹⁴ and decreases fibrinogen concentration and platelet counts.^{15,16} Acidification of human plasma with hydrochloric acid impairs coagulation as measured by thromboelastography (TEG).¹⁷ These results suggest that acidosis can lead to a decrease in coagulation function. Furthermore, if hemorrhagic shock and trauma are associated with acidosis, and if acidosis suppresses coagulation function, then it may be expected that correction of the acidosis would also correct the coagulopathy seen in hemorrhagic shock and trauma.

Few reliable models of hemorrhagic shock have been able to achieve an arterial pH ≤ 7.1 before the animal succumbs to the blood loss. Therefore, many studies have used the infusion of HCl to achieve experimental acidosis.^{14–16} In our interest to understand the lethal triad of hypothermia, acidosis, and coagulopathy and to investigate possible treatment strategies, the purpose of this study was to develop a reproducible model of acidosis that is associated with hemorrhagic shock and to compare the coagulation changes with HCl-infused acidosis. Furthermore, we determined whether correction of the acidosis with bicarbonate (return arterial pH to 7.4) would rapidly restore normal coagulation function.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use Committee of the US Army Institute of Surgical Research. All animals received care and were used in strict compliance with the Guide for the Care and Use of Laboratory Animals.¹⁸

Yorkshire cross-bred immature female pigs weighing 40.3 kg \pm 0.3 kg were purchased from Midwest Research Swine (Gibbon, MN) and used in these studies. Before

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surgery, venous blood samples were collected from pigs, and complete blood count and the coagulation profile (prothrombin time [PT], activated partial thromboplastin time [aPTT], and fibrinogen concentration) were measured to ensure that these values were within the normal range before proceeding with experimentation. On the day of surgery, pigs were premedicated with buprenorphine (0.01 mg/kg, intramuscularly [i.m.]) for analgesia and glycopyrrolate (0.01 mg/kg, i.m.) to reduce saliva secretion and block vagally mediated bradycardia during the surgical procedure. Animals were then induced with Telazol (4–6 mg/kg, i.m.), anesthetized with isoflurane, and intubated and mechanically ventilated with room air and oxygen mixture ($\text{FiO}_2 = 0.31$) for the hemorrhage/hypoventilation group and with 100% O_2 for the HCl-infusion group.

Before surgery, the tidal volume and ventilation rate were adjusted to maintain an end-tidal pCO_2 of 40 mm Hg \pm 5 mm Hg. Anesthesia was maintained with 0.8% and 1.5% isoflurane added to air/oxygen by the ventilator. Maintenance fluid, lactated Ringer's, was administered at 5 mL/kg/hr through a venous line placed in an ear vein, and no heparin or other anticoagulant was used to keep the lines open. Core temperature was maintained at 38°C.

The right carotid artery was cannulated for the direct measurements of blood pressure (systolic, diastolic, and mean arterial pressure [MAP]) and heart rate throughout the experiment. The right external jugular vein and right femoral vein were catheterized. Venous blood samples were taken from the jugular vein. Administration of drugs or fluids was via the right femoral vein. The right femoral artery was cannulated for withdrawal and reinfusion of blood. Arterial blood samples were taken from the carotid artery. At the end of the experiment, all pigs were killed with 10 mL of Fatal-Plus intravenously (i.v.).

Two separate experiments were performed. In the first experiment, acidosis (arterial pH 7.1) was induced in the pigs by infusing 0.2 mol/L HCl solution i.v. ($n = 10$). The second experiment used a combination of controlled hemorrhage and decrease in respiration ($n = 8$) to achieve the same pH level as in the HCl infusion study.

To determine the effects of acidosis on coagulation in both experiments, we measured various coagulation parameters (as described below) before infusion of HCl or hemorrhage at pH of 7.4 (Baseline), at a pH of 7.1 (Acidosis), and after correction of pH to 7.4 with injection of bicarbonate (Acidosis-Correction). A timeline of this experiment is schematically represented in Figure 1 for both groups.

HCl-Induced Acidosis

Acidosis was induced by infusion of a solution of HCl in saline (0.2 mol/L, i.v.) at 2 mL/min to 10 mL/min until a pH of 7.1 was reached as previously described.^{15,16} The amount of HCl solution needed to lower pH was variable from pig to pig (1,808 mL \pm 187 mL) and was infused over 268 minutes \pm 30 minutes. After pH 7.1 stabilized (10–15 minutes after HCl infusion), another blood sample was taken for determination of coagulation parameters. Approximately 5 minutes after this blood sample was taken, sodium bicarbonate (8.4% solution, Hospira, Inc., Lake Forest, IL) was

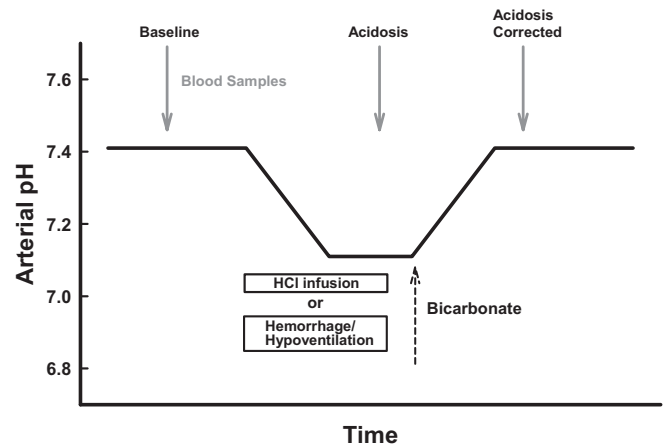


Figure 1. Time line showing the relationship between blood sampling (Baseline, Acidosis, and Acidosis-Corrected), induction of acidosis by either the hemorrhage/hypoventilation or the HCl-infusion groups, and correction of acidosis with bicarbonate. Total experiment time and time between Baseline, Acidosis, and Acidosis-Corrected varied from pig to pig. y axis describes the pH of the swine blood at the time of sampling.

infused i.v. (280 mL \pm 21 mL) until arterial pH stabilized at 7.4. After 10 minutes to 15 minutes, a final blood sample was taken for determination of coagulation parameters (Correction). Blood samples were collected from the arterial line at baseline, at pH 7.1, and at pH 7.4 (Fig. 1) for measurement of coagulation parameters as described below.

Hemorrhage/Hypoventilation-Induced Acidosis

After completion of surgery, 100 mg/kg/min to 500 mg/kg/min of ketamine was infused via the femoral vein and the isoflurane level reduced to 1%. This plane of anesthesia completely stopped the voluntary breathing of the pig, and respiration was completely controlled by the ventilator.

Animals were then subjected to a controlled hemorrhage at 50 mL/min to a target MAP of 30 mm Hg and held at that pressure by continuous bleeding or infusion of shed blood. During hemorrhage, blood was collected in citrate phosphate dextrose blood bags and stored at 37°C for reinfusion to maintain MAP at 30 mm Hg. Severe hemorrhagic shock was accomplished by withdrawing blood until MAP decreased to 30 mm Hg and was held at 30 mm Hg by further blood withdrawal or reinfusion of blood collected in blood bags. The volume of blood removed (1,454 mL \pm 51 mL) and reinfused (791 mL \pm 94 mL) varied from pig to pig. By the end of the experiment, the total amount of blood lost after hemorrhage and reinfusion was 664 mL \pm 99 mL. Blood samples (1.5 mL in lithium heparin) were taken from the carotid artery every 15 minutes for monitoring of arterial blood gasses, plasma chemistry, Ca^{++} , and pH using an IRMA TruPoint Blood Analysis System (International Technidyne Corporation, Edison, NJ). Arterial pH was measured every 15 minutes, which fell initially during hemorrhage and then reached a plateau level of 7.33 ± 0.02 at 107 minutes \pm 7 minutes posthemorrhage. At this point, ventilation was

adjusted (lowering tidal volume and/or ventilation rate) until arterial pH fell to and remained at 7.1 (Fig. 1). When arterial pH stabilized at 7.1 for 10 minutes to 15 minutes, a blood sample was taken (Acidosis) for determination of coagulation parameters. Approximately 5 minutes after this sample was taken, 100 mL of sodium bicarbonate (8.4% solution) was infused i.v. over a period of 1 minute. Simultaneously, ventilation (tidal volume/ rate) was adjusted to prehemorrhage levels. When pH stabilized at 7.4 for 10 minutes to 15 minutes, a final blood sample was taken (Acidosis-Correction). Blood samples were collected from the arterial line at baseline, at pH 7.1 and at pH 7.4 (Fig. 1) for measurement of coagulation parameters as described below.

Plasma fibrinogen concentration, PT, and aPTT were measured using the BCS Coagulation System (Dade Behring, Deerfield, IL). Platelet counts were measured using an ABX Pentra 120 Hematology Analyzer (ABX Diagnostics, Irvine, CA).

TEG was run immediately after blood samples were taken (no anticoagulant) using a TEG Hemostasis Analyzer 5000 (Hemoscope, Niles, IL). The accuracy of the TEG machines was checked daily using quality control standards obtained from Hemoscope. For this assay, clotting was initiated by adding 10 μ L of human recombinant tissue factor (diluted 1:200 with saline, Innovin) to 340 μ L of fresh blood samples and the clotting profile traced. Samples were measured in triplicate, and the tracing continued for at least 30 minutes after the clot reached maximum strength. The following variables were measured for each sample at the animal's normal body temperatures (39°C): reaction time (*R*, minutes, the time for initial detection of fibrin formation); clotting time (*K*, minutes, the time from the *R* time until a clot with a fixed firmness is formed); angle (α , degree, the kinetics of clot development); and maximum amplitude (millimeter, the maximum strength or firmness of the developed clot). Activated clotting time (ACT) was measured in fresh whole blood (Hemochron, International Technidyne Corporation, Edison, NJ) by manufacturer's instructions.

The thrombin generation assay was run on aliquots of citrated plasma that were frozen at -70°C and assayed in batches at later dates. Thrombin generation was measured by a Calibrated Automated Thrombinoscope (Fluoroskan Ascent; ThermoScientific, Waltham, MA) in platelet-poor plasma (80 μ L) mixed with a reagent containing phospholipids and tissue factor in 96-well plates. Added to this mixture was a buffer containing substrate that was enzymatically cleaved by thrombin to produce a fluorescent product. Calcium chloride was added to neutralize anticoagulants. Thrombograms were used to determine endogenous thrombin potential (ETP), maximum thrombin concentration (peak), time to peak (ttPeak), and lag time (time to start of thrombin formation) for each sample. All assays were performed in triplicate.

Data were analyzed by one- or two-way analysis of variance corrected for repeated measure followed by multiple comparison analysis compared with baseline using the Holm-Sidak method. If the normality test failed, then Friedman analysis of variance on Ranks was performed, followed by

TABLE 1. HCl Group

n = 10	Baseline	Acidosis (HCl infusion)	Correction (Bicarbonate)
pH	7.40 \pm 0.01	7.13 \pm 0.1*	7.38 \pm 0.01
PaCO ₂ (mm Hg)	55.2 \pm 0.8	48.2 \pm 3.1*	55.0 \pm 3.2
PaO ₂ (mm Hg)	479.8 \pm 26.2	464.5 \pm 26.1	455.4 \pm 16.6
HCO ₃ ⁻ (mmol/L)	33.2 \pm 0.7	16.2 \pm 1.8*	31.4 \pm 1.5
BE (mmol/L)	7.0 \pm 0.7	-12.5 \pm 2.2*	5.2 \pm 1.2

* Significant difference ($p < 0.05$) from baseline by one-way analysis of variance corrected for repeated measure with pairwise multiple comparison post hoc test (Holm-Sidak method).

Values represent mean \pm SEM.

multiple comparisons versus baseline by Dunn's method. Statistics were performed using SigmaStat Software. Data are expressed as mean \pm SEM, and $p < 0.05$ was considered significant.

RESULTS

Acidotic Models

Both swine models of acidosis were used successfully to investigate changes in coagulation function. Infusion of HCl reduced arterial pH from 7.4 to 7.1 and also reduced HCO₃⁻, base excess (BE), and PaCO₂ (Acidosis, Table 1). In this group, bicarbonate infusion returned arterial pH to 7.4, and HCO₃⁻, BE, and PaCO₂ to preacidosis levels (Acidosis-Correction, Table 1). HCl infusion also lowered mean arterial blood pressure from 78 mm Hg \pm 3 mm Hg to 60 mm Hg \pm 3 mm Hg. Bicarbonate infusion did not significantly change arterial pressure.

Severe hemorrhagic shock, by itself, failed to lower arterial pH below 7.3, although arterial HCO₃⁻ and BE fell significantly (Table 2). Combining blood loss with a decrease in respiration successfully lowered arterial pH to 7.1 (Acidosis, Table 2) and significantly elevated PaCO₂ and HCO₃ and lowered Pao₂ (Table 2). Bicarbonate infusion together with normalization of respiration returned arterial pH to 7.4 (Acidosis-Correction, Table 1), and returned PaCO₂, Pao₂, and BE to near their prehemorrhage levels. CaCl₂ was injected i.v. during reinfusion of citrated blood (1 mg/mL of blood), so that the plasma Ca⁺⁺ would not fall below 1 mmol/L and hinder the ability of the blood to clot. Table 3 shows that Ca⁺⁺ remained >1.2 mmol/L throughout the experiments. Hemorrhage/hypoventilation also led to a significant rise in plasma lactate and potassium and a fall in glucose concentration (Table 3), which is indicative of the severity of this shock model.

Acidosis-Induced Coagulopathy

HCl-induced acidosis significantly elevated PT, aPTT, and ACT as compared with baseline (Fig. 2). However, hemorrhage/hypoventilation-induced acidosis did not affect PT, aPTT, or ACT values (Fig. 2). Correction of pH with bicarbonate did not correct PT, aPTT, or ACT in the HCl group, and these parameters remained significantly elevated above normal levels (Fig. 2).

TABLE 2. Hemorrhage/Hypoventilation Group

n = 8	Before Hemorrhage (Baseline)	Hemorrhage	Hemorrhage/Hypoventilation (Acidosis)	Normal Ventilation + Bicarbonate (Correction)
pH	7.41 ± 0.01	7.33 ± 0.02*	7.11 ± 0.01*†	7.42 ± 0.01
PaCO ₂ (mm Hg)	49.3 ± 1.8	45.4 ± 1.3	89.2 ± 4.5*†	49.7 ± 2.2
PaO ₂ (mm Hg)	143.5 ± 9.6	138.0 ± 11.9	99.1 ± 9.8*†	162.4 ± 7.5
HCO ₃ ⁻ (mmol/L)	30.5 ± 1.3	22.6 ± 1.2*	27.2 ± 1.4†	30.1 ± 1.3
BE (mmol/L)	5.2 ± 1.0	-2.9 ± 1.3*	-4.5 ± 1.1*	5.9 ± 1.2

* Significant difference ($p < 0.05$) from baseline.

† Significant difference between Hemorrhage and Acidosis, by one-way analysis of variance corrected for repeated measure with pairwise multiple comparison post hoc test (Holm-Sidak method).

Values represent mean ± SEM.

TABLE 3. Plasma Chemistry in Hemorrhage Group

	Baseline	Acidosis	Correction
Ca ⁺ (mmol/L)	1.37 ± 0.03	1.37 ± 0.02	1.24 ± 0.02
Na (mmol/L)	138.4 ± 0.4	139.3 ± 0.6	139.9 ± 0.5
K (mmol/L)	4.00 ± 0.06	5.61 ± 0.31*	6.71 ± 0.36*†
Cl (mmol/L)	98.1 ± 0.6	98.9 ± 0.5	96.8 ± 0.6*
Glucose (mmol/L)	4.13 ± 0.48	2.23 ± 0.49*	1.58 ± 0.34*
Lactate (mmol/L)	1.61 ± 0.19	6.48 ± 1.16*	7.15 ± 1.54*

* Significant difference ($p < 0.05$) from baseline.

† Significant difference between Acidosis and Correction. Analyzed by one-way analysis of variance corrected for repeated measure with pairwise multiple comparison post hoc test (Holm-Sidak method). Plasma chemistry was not measured in the HCl infusion study.

Values represent mean ± SEM.

TEG analysis of whole blood taken from pigs made acidotic by either HCl infusion or hemorrhage/hypoventilation showed a decrease in coagulation status as reflected in all TEG parameters (Fig. 3). In both groups, there was a significant decrease in maximum amplitude and α angle when pH reached 7.1. Reaction time (R) and clotting time (K) rose in both groups; however, this rise was not statistically significant in the hemorrhage/hypoventilation group because of large variability. Returning pH to 7.4 with bicarbonate did not restore the TEG coagulation parameters to baseline levels (Fig. 3), and the hypocoagulation was not corrected in either group.

The thrombin generation assay also showed that acidosis led to a hypocoagulation (Fig. 4). HCl infusion significantly prolonged both Lagtime and ttPeak. Hemorrhage/hypoventilation only prolonged ttPeak. Acidosis also significantly decreased thrombin Peak and ETP in both groups. Correction of pH with bicarbonate restored only ttPeak in the hemorrhage/hypoventilation group. In both groups, thrombin Peak and ETP remained decreased even after pH correction.

Fibrinogen concentration and platelet counts fell significantly after induction of acidosis in both groups (Fig. 5). However, infusion of HCl or severe hemorrhage/hypoventilation did not affect hematocrit (Fig. 5). The infusion of HCl caused a significantly greater fall in both fibrinogen concentration ($44.3\% \pm 2.8\%$) and platelet count ($33.6\% \pm 6.1\%$) than occurred with hemorrhage ($14.5\% \pm 5.5\%$ and $14.1\% \pm 4.1\%$, respectively), despite removal of a significant volume

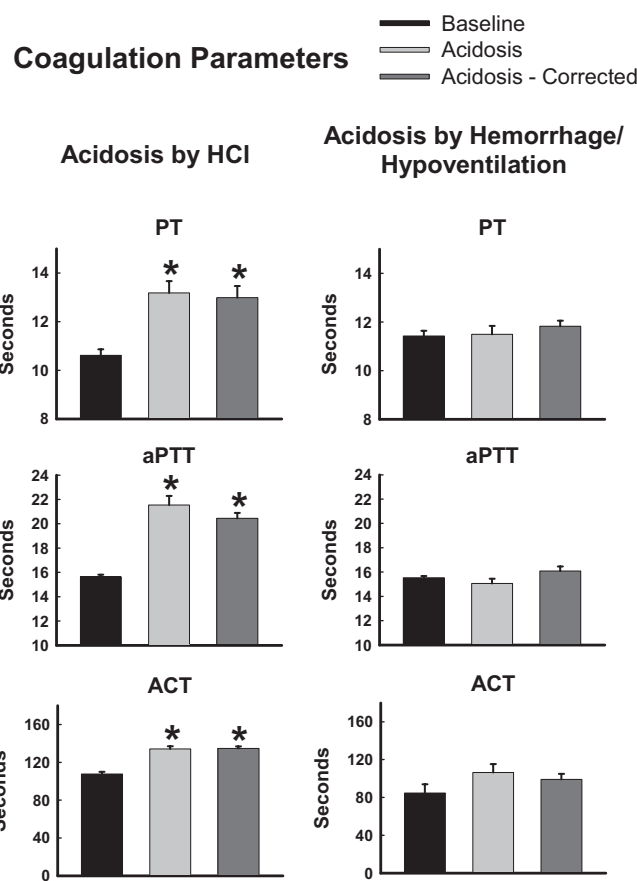


Figure 2. Plasma clotting values from whole blood and plasma collected from pigs made acidotic by either infusion of 0.2 mol/L HCl or hemorrhage/hypoventilation. PT, prothrombin time; aPTT, activated partial thromboplastin time; ACT, activated clotting time. * $P < 0.05$ as compared to baseline. Values = mean ± SEM.

of blood. Correction of the acidosis with bicarbonate did not affect fibrinogen concentration or platelet counts in the HCl group, and both parameters remained significantly lower than baseline (Fig. 5).

DISCUSSION

This study demonstrates that acidosis caused by either hemorrhage/hypoventilation or HCl infusion leads to a

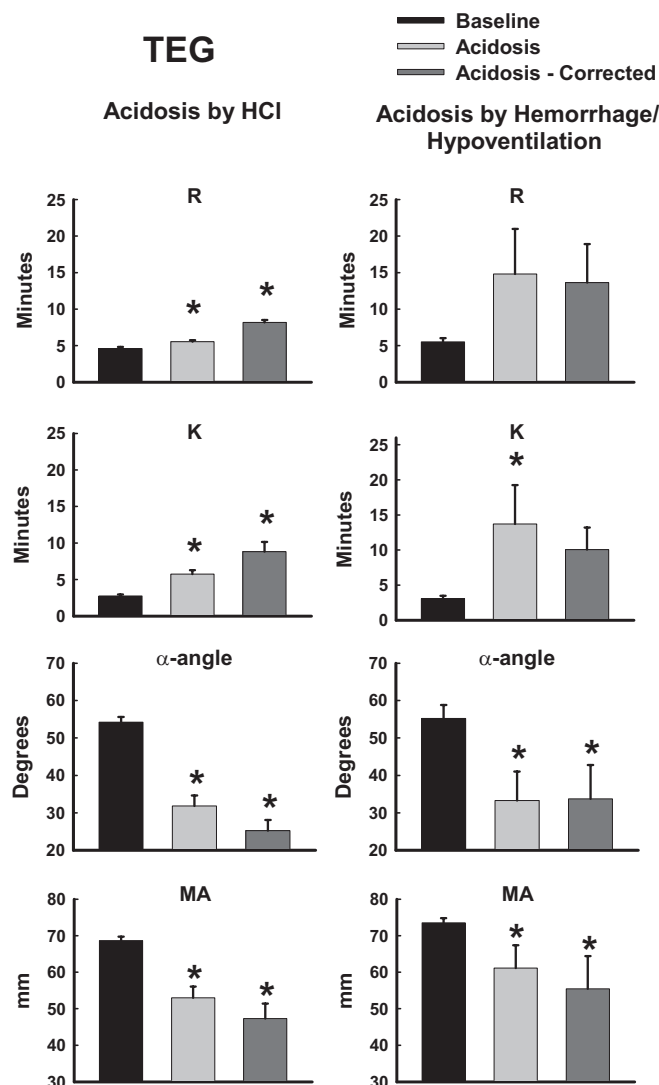


Figure 3. TEG parameters from whole blood collected from pigs made acidotic by either infusion of 0.2 mol/L HCl or hemorrhage/hypoventilation. R, reaction time; K, clotting time; angle, rate of clot formation, MA, maximum clot strength. * $P < 0.05$ as compared to baseline. Values = mean \pm SEM.

decrease in coagulation function. Furthermore, correction of the acidosis with bicarbonate infusion did not immediately correct or alleviate this coagulopathy. At present, the mechanisms associated with these observations, such as consumption or pH-induced alterations of coagulation factors or platelets, loss of factors, changes in enzyme function, or a combination of processes, are unknown and cannot be reversed by pH correction alone. Future studies that focus on the time course of these processes are needed to explain this phenomenon.

There were some differences in the coagulation results obtained with the two models to induce acidosis. HCl infusion led to a significant delay in the clotting reaction as measured by increases in PT, aPTT, ACT, and Lagtime. This

Thrombin Generation

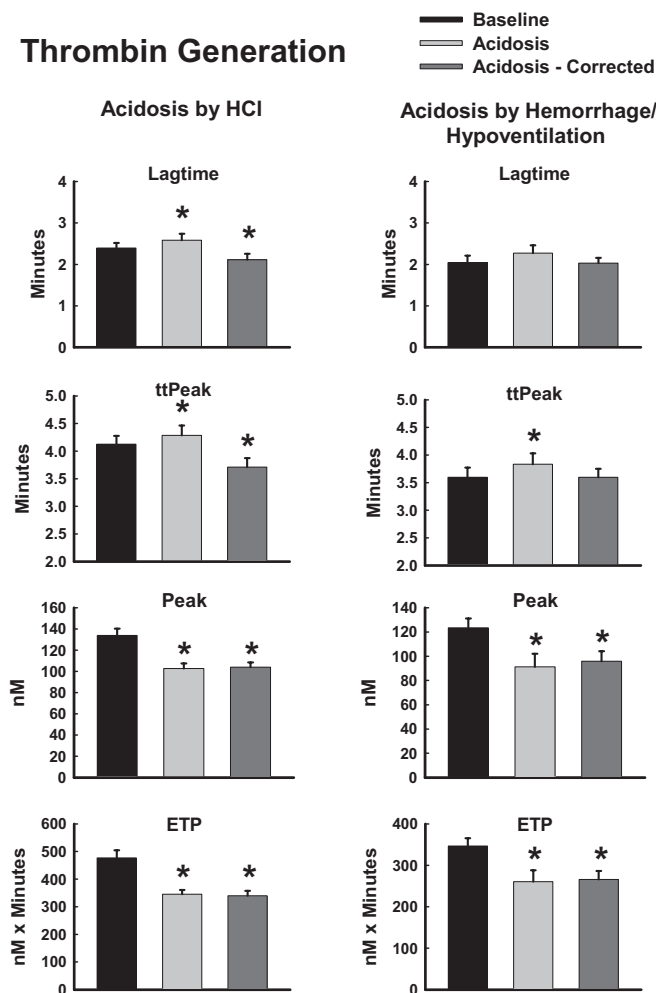


Figure 4. Thrombin generation assay from plasma collected from pigs made acidotic by either infusion of 0.2 mol/L HCl or hemorrhage/hypoventilation. Lagtime, time to start of thrombus formation; ttPeak, time to Peak; Peak, maximum thrombin concentration; ETP, endogenous thrombin potential. * $P < 0.05$ as compared to baseline. Values = mean \pm SEM.

did not occur in the hemorrhage/hypoventilation group and highlights the biggest differences between the two acidosis models. The variability in the TEG data was greater in the hemorrhage/hypoventilation group, although the changes in the clotting parameters were similar and in the same direction for both models.

The hypocoagulation that was measured by TEG and ACT could be partially explained by the decreases in the fibrinogen concentration and platelet count. Fibrinogen is a key coagulation substrate that is converted by thrombin to form a fibrin gel that then undergoes covalent cross-linking catalyzed by factor XIIIa to further strengthen the clot. A decrease in fibrinogen concentration can lead to a decreased rate of clot formation and clot strength. Platelets play an important role in primary hemostasis, as they adhere and aggregate to the site of injury and provide a surface for

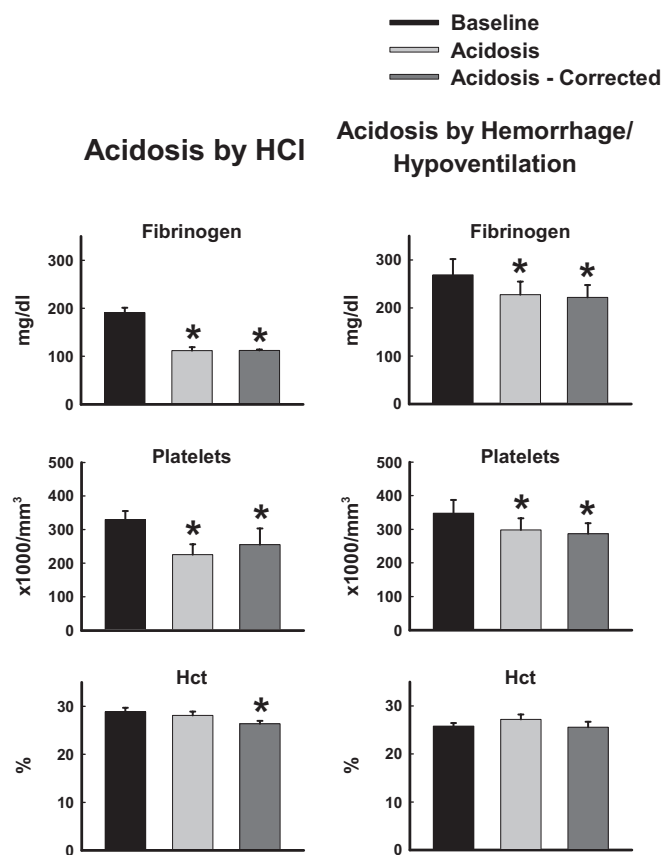


Figure 5. Fibrinogen concentration, platelet count, and Hematocrit (Hct) from blood collected from pigs made acidotic by either infusion of 0.2 mol/L HCl or hemorrhage/hypoventilation. * $P < 0.05$ as compared to baseline. Values = mean \pm SEM.

thrombin generation and enhance clot formation. A decrease in platelet count would affect coagulation assays like TEG and ACT that use whole blood rather than plasma.

In this study, we found that acidosis decreased both ETP and thrombin peak as measured by the thrombin generation assay. A decrease in thrombin generation has also been seen in other acidosis studies^{14,15,19} in which thrombin was measured as thrombin-antithrombin III complex. These reports and the present study show that HCl infusion produces not only an acidosis but also a fall in both fibrinogen concentration and platelet count^{15,16} and possibly in other clotting factors as indicated by the prolonged PT and aPTT. The fall in fibrinogen concentration (Fig. 5) could be explained, at least in part, by protein hydrolysis caused by HCl infusion.

Severe blood loss leads to a movement of fluid from the extravascular space into the vascular space because of a decrease in capillary hydrostatic pressure, a mechanism originally defined by Starling.^{20,21} Restitution of plasma volume can be rapid in conscious rats and can restore plasma volume in 20 minutes to 40 minutes.^{22–24} Fluid that is mobilized to restore blood volume also comes from the interstitial space and within the alimentary tract.²² Fluid movement into the vascular space from the interstitial space may partially ex-

plain the decrease in platelet count and fibrinogen concentration in the hemorrhage/hypoventilation group. However, no significant changes in hematocrit were observed in this study. Because the pig has a contractile spleen, which can significantly add to its blood volume, it is possible that autotransfusion helped maintain hematocrit in these animals. However, in the hemorrhage/hypoventilation group, there was a 14% drop in platelets and fibrinogen levels, despite the maintenance of hematocrit. This suggests that other factors such as consumption or loss caused by acidosis may be involved, but this would require additional study. In the HCl infusion model, hemodilution could be expected to occur because of the large volume of fluid infused. It should be noted that HCl was infused over a long period of time (268 minutes \pm 30 minutes), thereby allowing sufficient time for renal excretion of the excess fluid and maintenance of hematocrit at normal levels. However, platelets and fibrinogen levels were reduced to a greater extent in this model compared with the hemorrhage/hypoventilation model. This was also observed previously,¹⁵ and because a similar volume of lactated Ringer's solution did not induce such a change, it was concluded that the observations were the result of the acidosis. Considering the important roles of platelets and fibrinogen in the coagulation process, the mechanisms to understand these reductions warrants further investigation.

Patients who have undergone severe trauma have been described as having a coagulopathic condition that can contribute to morbidity and mortality.²⁵ Hypocoagulation has been reported to be caused by multiple factors including consumption of clotting factors, dilution of plasma from resuscitation by crystalloids, acidosis, and hypothermia. It is evident that the hypocoagulation that is seen in trauma patients with hemorrhagic shock can be caused by one or multiple factors. Clinically addressing this coagulopathy will require a multifactorial approach that may include not only pH correction but also replenishment of blood components such as clotting factors, plasma, platelets, and fibrinogen.

Although the HCl model has been used several times and has been described as a model of hyperchloremic acidosis,^{15,16} its use has not been without controversy. HCl infusion leads to both an elevation in Cl^- and H^+ concentration. It is not known how or whether hyperchloremia, by itself, can affect coagulation. However, because the hemorrhage/hypoventilation model shows a similar coagulopathy as the HCl model, and does not lead to hyperchloremia (Table 3), it is suggestive that the rise in H^+ concentration, and not the rise in Cl^- concentration, is affecting coagulation in the HCl model. Further studies using Cl^- solutions with no change in pH could be used to determine whether elevated Cl^- itself, independent of pH, affects coagulation parameters.

This study also used a hemorrhage/hypoventilation model to develop metabolic acidosis in a model more relevant to trauma. Significant blood loss coupled with hypoventilation was required to develop a metabolic acidosis, as indicated by the increased base deficit. Hypoventilation and respiratory depression has been observed in trauma patients and can be caused by a decrease in neurologic function and/or mechanical obstruction caused by fluid or debris in the

lungs.²⁶ Our hemorrhage/hypoventilation model caused a very severe acidotic and hypovolemic condition, as failure to reverse the acidosis or maintain the animal's hypotension longer would have resulted in high mortality rates.

CONCLUSIONS

We have shown that acidosis associated with hemorrhagic shock or induced by HCl infusion leads to reduced coagulation function. Furthermore, simply correcting the acidosis with bicarbonate injection and normal ventilation did not restore normal coagulation rapidly in either the hemorrhage/hyperventilation or HCl infusion studies. These findings are of clinical importance and suggest that simple correction of the acidosis with bicarbonate is not sufficient to correct the coagulopathy associated with the lethal triad seen in patients with hemorrhagic shock, immediately. These data suggest that a multifactorial approach is necessary to correct the coagulopathy. Further work is necessary to determine whether more optimal use of blood products, such as plasma, coagulation factors, and fibrinogen, used alone or in combination, is required to correct coagulopathy associated with metabolic acidosis in trauma patients.

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